Effects of *Ganoderma Lucidum* sporoderm-broken spore extracts on proliferation and apoptosis in prostate cancer PC-3 cells

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ABSTRACT

Ganoderma Lucidum (Linzhi or Reishi), the most popular mushroom used in Traditional Chinese Medicine (TCM), has been used in Asian countries over thousand years for the treatment and prevention of many diseases, including cancer. However, the molecular mechanisms responsible for the anti-cancer effects of G.Lucidum remain to be elucidated. The objective of the current study was to determine the effects of the sporoderm-broken spore water extracts (SBSWE) of G.Lucidum on cell proliferation, apoptosis and the molecular targets in prostate cancer PC-3 cells. Our data demonstrated that the SBSWE (1.25 mg/ml - 10 mg/ml) significantly inhibited the proliferation of PC-3 cells in both a time- and dose-dependent manner. SBSWE treatment at 10 mg/ml for 72 hr produced maximal inhibitory effect on the PC-3 cell growth by 85% compared to the control. Western blot revealed that the growth inhibitory effects were associated with activation of caspase-9, caspase-3, caspase-6, and the cleavage of the poly (ADP-ribose) polymerase (PARP) in PC-3 cells. In addition, the phosphorylation of the extracellular signal-regulated kinase (Erk) was significantly inhibited by SBSWE in both a time- and dose-dependent manner. Phosphorylated Akt was also downregulated by SBSWE treatment in PC-3 cells. However, the activity of p38 was significantly upregulated by SBSWE treatment in these cells. We also determined that SBSWE were able to inhibit the growth of the normal human prostate epithelial isolate PrEC and the prostate non-tumorigenic RWPE-1 cells in a dose-dependent manner. Taken together, these observations suggest that the SBSWE of G.Lucidum contain very potent phytochemicals which effectively inhibited the growth of prostate PC-3 cells by targeting multiple signaling pathways.

HYPOTHESIS

An aqueous extract of sporoderm-broken spores of *G. Lucidum* will have anti-proliferative and pro-apoptotic effects on human prostate cancer cells.

OBJECTIVE

- To determine the effect of sporoderm-broken spore water extracts on cell proliferation in prostate cancer and non-cancer cells.
- To examine the mechanisms by which the sporoderm-broken spore water extracts regulate the growth of human prostate cells.

INTRODUCTION

Prostate cancer is the most common malignancy found in males and the second leading cause of cancer death in US men. Therefore, identification of promising chemopreventive agents may have a significant impact on public health.

G. Lucidum (Linzhi or Reishi), is the most popular mushroom used in Traditional Chinese Medicine (TCM) for over 2000 years for the promotion of vitality and longevity, and is hypothesized to have anti-cancer activities[1].

PC-SPES is an herbal mixture previously marketed by Botanic Lab (Brea, LA) from 1966 to 2002 and used by patients with prostate cancer2. G. Lucidum is one of the eight herbs used in PC-SPES. PC-SPES was successfully tested with promising results in phase II clinical trials as an effective agent in the treatment of advanced prostate cancer[2,3]. However, PC-SPEC was withdrawn from the market in 2002 after being found to have been supplemented with estrogens, anticoagulants and analgescs[2,3]. Although published research with PC-SPES is invalid, further research focusing upon the individual components, such as G. Lucidum are of critical importance. The water extracts of the sporoderm-broken spores of G. Lucidum contain bioactive components, such as polysaccharides, that maybe responsible for the anti-cancer activity of G. Lucidum [4]. We have conducted a series of studies to determine the potential of this agent for future development as a chemopreventive agent or adjunct to prostate cancer therapy.

METHODS

Materials. The sporoderm-broken spore product of G. Lucidum was provided as a kind gift from Chinese Academy of Science. All the antibodies were purchased from Cell Signaling (Beverly, MA). The 3-[4,5-2-yl]-2-, 5-diphenyltetrazolium bromide (MTT) were obtained from Sigma (St. Louis, MO).
Water extraction. The sporoderm-broken spores of G. Lucidum (100g) were extracted with 2 L of water at 70º C in a water bath with agitation for 12 h. The aqueous fraction obtained from centrifugation was lyphilized with Labconco Lyph-Lock 4.5 Freeze Dry System (Corona, CA) and stored at -20º C. The freeze-dried sporoderm-broken spore water extracts (SBSWE) were then dissolved in appropriate medium at indicated concentrations for subsequent experiments.
Cell Proliferation Assay. Cells were plated in 96-well microtiter plates at an initial density of 2 x 104 cells per well. Cells were treated with SBSWE (0, 1.25, 2.5, 5, and 10 mg/ml) and incubated for an additional 24, 48 or 72 h. After incubation, cell proliferation was determined by the MTT assay according to manufacturer's instruction (Sigma, MO).
Cell culture. Human prostate non-cancerous RWPE-1 and cancerous PC-3 cells were purchased from ATCC (Rockville, MD). Normal human prostate epithelial cells (PrEC) were purchased from Clonetics (Walkersville, MD). All cells were maintained in appropriate growth medium at 37º C in 5% CO2.
Immunoblotting. Cells were grown and treated with SBSWE in 35-mm dishes (75% confluent). After appropriate treatments, cell lysates were collected and protein concentrations were measured. Approximately 30 µg protein was electrophoresed on a 10% SDS-polyacrylamide gel followed by standard immunoblotting procedures according to manufacturer's instruction (Cell Signaling, MA).

RESULTS

Effect on Prostate Cell Growth

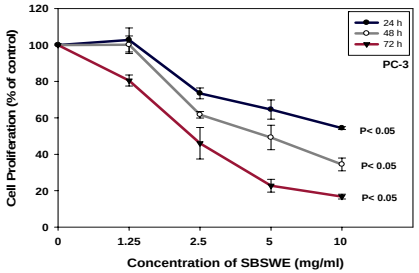

Figure 1. Dose- and time-dependent inhibition of the growth of prostate cancer PC-3 cells by SBSWE. PC-3 cells were treated with SBSWE (0 – 10 mg/ml) for 24, 48 and 72 h followed by MTT assay. All experiments were repeated three times in duplicate. Data represent Mean + SD. A p-value of < 0.05 is considered as significant.

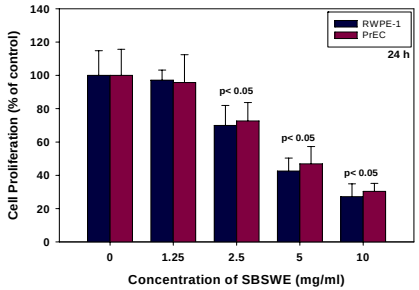

Figure 2. Dose-dependent inhibition of the growth of prostate non-cancerous PWPE-1 and normal PrEC cells by SBSWE. RWPE-1 and PrEC cells were treated with SBSWE (0 – 10 mg/ml) for 24 h followed by MTT assay. All experiments were repeated three times. Data are represented as Mean ± SD. A p-value of < 0.05 is considered as significant.

Effect on MAPKs and Akt Signaling

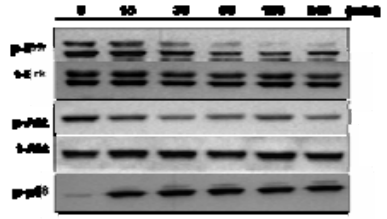

Figure 3. Time-dependent regulation of MAPKs and Akt signaling in PC-3 cells by SBSWE. PC-3 cells were treated with SBSWE at 10 mg/ml for indicated time points (0 – 240 min). Cell lysates were collected and analyzed by immunoblotting for phosphorylated Erk (p-Erk), total Erk (t-Erk), phosphorylated Akt (p-Akt), total Akt (t-Akt) and phosphorylated p38 (p-p38).

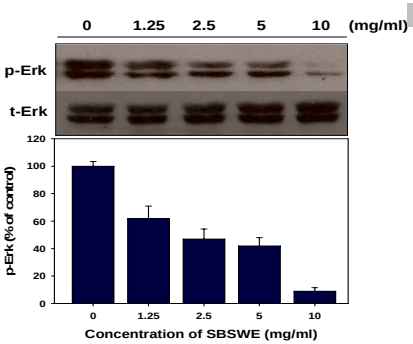

Figure 4. Dose-dependent regulation of Erk phosphorylation in PC-3 cells by SBSWE. PC-3 cells were treated with SBSWE (0 – 10 mg/ml) for 1 h. Cell lysates were collected and analyzed by immunoblotting for phosphorylated Erk (p-Erk) and total Erk (t-Erk). One representative blot and its quantification (by Scion Image Software) are given.

Effect on apoptotic Signaling

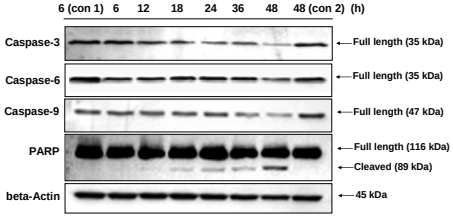

Figure 5. Time-dependent regulation of caspase and PARP cleavage in PC-3 cells by SBSWE. PC-3 cells were treated with SBSWE at 10 mg/ml for indicated time points (6 – 48 h). Control 1 (Con 1) was collected at 6 h without treatment. Control 2 (Con 2) was collected at 48 h without treatment. Cell lysates were collected and analyzed by immunoblotting for full length caspase -3, -6, -9 and PARP.

Working Model

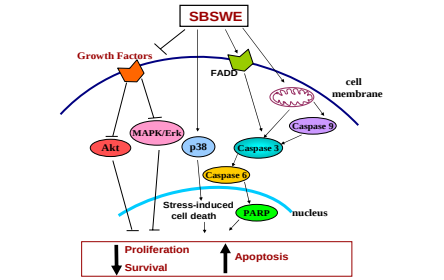

Figure 6. Schematic drawing of the mechanisms by which SBSWE of *G. Lucidum* regulate the growth of prostate cancer PC-3 cells. SBSWE may directly activate the apoptosis pathway by activating the death receptor FADD or the mitochondrial-mediated signalings. SBSWE may also inhibit cell proliferation and survival though the inhibition of MAPK/Erk and Akt pathways.

CONCLUSIONS

- Water extracts of the sporoderm-broken spores of *G. Lucidum* inhibite the growth of human prostate cancer PC-3 cells in a time- and dose-dependent manner.
- Water extracts of the sporoderm-broken spores of *G. Lucidum* inhibit growth promoting and survival pathways involving MAPKs, Akt, caspases, and PARP.

FUTURE DIRECTIONS

- To further define the effects of SBSWE on cellular and molecular pathways involved in growth inhibition and activation of apoptotic pathways in prostate cancer cells.
- To determine the effects of SBSWE on prostate tumorigenesis and carcinogenesis *in vivo*.
- To isolate and quantitate the bioactive components in SBSWE of *G. Lucidum*.

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In memorium (1962-2006): Russell D. Klein, Ph.D passed away on December 1, 2006 after a year long battle with leukemia at the James Cancer Hospital. He will be greatly missed by his colleagues and students as a superb scientist, mentor, and gentleman.

References:
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